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| (51) International Patent Classification <sup>6</sup> :<br>A61K 9/14, 9/50, B01J 13/00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |               | A1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | (11) International Publication Number: <b>WO 99/20253</b>     |               |               |               |               |               |               |   |   |   |   |   |   |   |    |    |    |    |    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |
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| (21) International Application Number: PCT/SE98/01717<br>(22) International Filing Date: 24 September 1998 (24.09.98)<br>(30) Priority Data:<br>9703874-9      23 October 1997 (23.10.97)      SE<br>(71) Applicant (for all designated States except US): BIOGLAN THERAPEUTICS AB [SE/SE]; P.O. Box 50310, S-202 13 Malmö (SE).<br>(72) Inventors; and<br>(75) Inventors/Applicants (for US only): LAAKSO, Timo [SE/SE]; Boltenssternsväg 33D, S-236 38 Höllviken (SE). RESLOW, Mats [SE/SE]; Bondevägen 45, S-227 38 Lund (SE).<br>(74) Agent: AWAPATENT AB; P.O. Box 45086, S-104 30 Stockholm (SE).                                                                                                                                                                                                                                                                                                                                                                                         |               | (81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br>With international search report.<br>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. |                                                               |               |               |               |               |               |               |   |   |   |   |   |   |   |    |    |    |    |    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |
| (54) Title: ENCAPSULATION METHOD                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                               |               |               |               |               |               |               |   |   |   |   |   |   |   |    |    |    |    |    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |
| <table border="1"> <caption>Estimated data from Cumulative Release graph</caption> <thead> <tr> <th>Time (days)</th> <th>Example 1 (%)</th> <th>Example 2 (%)</th> <th>Example 3 (%)</th> <th>Example 4 (%)</th> <th>Example 5 (%)</th> </tr> </thead> <tbody> <tr><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr> <tr><td>1</td><td>35</td><td>85</td><td>60</td><td>65</td><td>25</td></tr> <tr><td>5</td><td>40</td><td>95</td><td>60</td><td>70</td><td>25</td></tr> <tr><td>10</td><td>50</td><td>98</td><td>60</td><td>72</td><td>35</td></tr> <tr><td>15</td><td>60</td><td>99</td><td>60</td><td>75</td><td>35</td></tr> <tr><td>20</td><td>70</td><td>100</td><td>60</td><td>75</td><td>50</td></tr> <tr><td>25</td><td>75</td><td>100</td><td>60</td><td>78</td><td>55</td></tr> <tr><td>30</td><td>80</td><td>100</td><td>60</td><td>80</td><td>65</td></tr> </tbody> </table>                                                                                     |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                               | Time (days)   | Example 1 (%) | Example 2 (%) | Example 3 (%) | Example 4 (%) | Example 5 (%) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 35 | 85 | 60 | 65 | 25 | 5 | 40 | 95 | 60 | 70 | 25 | 10 | 50 | 98 | 60 | 72 | 35 | 15 | 60 | 99 | 60 | 75 | 35 | 20 | 70 | 100 | 60 | 75 | 50 | 25 | 75 | 100 | 60 | 78 | 55 | 30 | 80 | 100 | 60 | 80 | 65 |
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| (57) Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                               |               |               |               |               |               |               |   |   |   |   |   |   |   |    |    |    |    |    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |
| <p>A novel method of encapsulating an active substance in a biodegradable polymer, which comprises: a) dissolving said biodegradable polymer in an organic solvent therefor; b<sub>1</sub>) dispersing said active substance in the organic solution obtained in step a), to provide a dispersion with the active substance as the inner phase thereof; or alternatively b<sub>2</sub>) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a), to provide an emulsion with the active substance as the inner aqueous phase thereof; and c) subjecting the dispersion obtained in step b<sub>1</sub>), or alternatively the emulsion obtained in step b<sub>2</sub>), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase, to provide micro- or nanoparticles having the active substance encapsulated therein. Sustained release particles obtainable therebys.</p> |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                               |               |               |               |               |               |               |   |   |   |   |   |   |   |    |    |    |    |    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |    |    |     |    |    |    |

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ENCAPSULATION METHODFIELD OF INVENTION

The present invention is within the field of encapsulating active substances, e.g. drugs, in biodegradable polymers. More specifically the invention relates to a novel advantageous encapsulation method which is suitable for water soluble as well as water insoluble active substances and which gives highly active micro as well as nanoparticles with high encapsulation efficiency.

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BACKGROUND OF THE INVENTION

The encapsulation of materials may provide beneficial properties. For example, drugs that are encapsulated may provide increased stability, longer duration of action and increased efficiency. For convenience drugs are often encapsulated in solid materials which have a size suitable for injection, that is generally below 200  $\mu\text{m}$  in diameter, and then the process is referred to as a microencapsulation.

Microencapsulation processes may yield microcapsules, microspheres or microparticles. Microcapsules consist of a core and a shell that covers the core. The core may be composed of another polymer than the shell or of another material altogether, e.g. of the active substance itself. The active substance is generally located in the core but may also be located in the outer shell. Microspheres are spherical in shape and have a more homogenous matrix. Microparticle is a more general term than microspheres in that it is not restricted to spherical shapes. Sometimes it can be difficult to distinguish between microcapsules, microspheres and microparticles, and the term microparticles will be used herein with reference to all three classes.

Methods of preparing microparticles in the prior art have been described extensively in both the patent and

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the scientific literature (see e.g. Jalil R, Nixon JR. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. *J Microencapsul* 5 1990;7:297-325). They may generally be classified in three types, which are exemplified below in connection with the preparation of microspheres of poly(lactide-co-glycolide) (PLGA). PLGA is a well accepted polymer for preparing sustained release microspheres and often the 10 first choice for preparing biocompatible microspheres intended for parenteral administration in humans. Said polymer is not soluble in water.

1. Phase separation techniques using coacervating agents, or non solvents, such as mineral oils and vegeta- 15 ble oils. The active substance, e.g. a polypeptide is first dissolved in the aqueous phase of a water-in-oil emulsion. The polypeptide can also be dispersed directly in the polymer phase as a fine powder. Polymer is precipitated either around the aqueous droplets, or on the 20 polypeptide powder, by the addition of a non-solvent for the polymer, such as silicon oil. Then a hardening agent is added to extract the organic solvent from the microspheres. The main disadvantage with said process is the large amount of organic solvent needed for extraction and 25 for washing. The previously used hardening agents including freons, hexane, heptane, cyclohexane and other alkane solvents leave substantial amounts of hardening agents residues in the microspheres and/or necessitate extensive procedures for removing the solvent. Often very large 30 amounts of the second organic solvent are needed and they are often undesirable for health, economical and environmental reasons. Examples in the prior art include heptan (EP 0 052 510), aliphatic fluorinated and fluorohalogenated hydrocarbons sold as FREONS (SE 462 780), and other 35 (US 5,000,886). A further drawback when using e.g. an alkane hardening solvent is that it is flammable. Another drawback is the impact thereof on the environment.

## 2. Spray drying and spray coating

In spray drying the polymer and the drug are mixed together in a solvent for the polymer. The solvent is then evaporated by spraying the solution. This results in polymeric droplets containing the drug. However, sensitive substances such as proteins can be inactivated during the process due to the elevated temperatures used and the exposure to organic solvent/air interfaces. Further disadvantages include generation of high porosity due to rapid removal of the organic solvent. A variation that has been introduced to avoid these shortcomings is the use of low temperature during microsphere formation (US 5,019,400, WO 90/13780 and US 4,166,800). Microcapsules have been prepared using spray coating of drug-containing microparticles with PLGA polymers (US 4,568,559).

## 3. Solvent evaporation

In solvent evaporation techniques the polymer is dissolved in an organic solvent which contain the dispersed active drug, the solution then being added to an agitated aqueous outer phase which is immiscible with the polymer. The aqueous outer phase usually contains surfactants to stabilise the oil-in-water emulsion and to prevent agglomeration. The emulsifier used is typically polyvinylalcohol. Emulsifiers are included in the aqueous phase to stabilise the oil-in-water emulsion. The organic solvent is then evaporated over a period of several hours or more, thereby solidifying the polymer to form a polymeric matrix. The solvent can also be extracted by adding the above mentioned suspension to a large volume of water (US 5,407,609).

The final formulation to be used for pharmaceutical applications, especially for parenteral administration, should consist of discrete, non-agglomerated microspheres with the desired size distribution and containing no toxic or in any other way undesirable substances. In order to obtain preparations having the characteristics described above it is necessary to use emulsifiers. The

emulsifier can serve several purposes: (1) assist in obtaining the correct droplet size distribution of the emulsion; (2) stabilise the oil-in-water emulsion to avoid coalescence of the droplets; and (3) prevent the solidified microspheres from sticking to each other. The most commonly used emulsifier for preparing PLGA microspheres is polyvinyl alcohol. However, since polyvinyl alcohol is listed in the 1976 Register of Toxic Effects of Chemical Substances and is also implicated as carcinogenic when introduced parenterally into animals ("Carcinogenic studies on Water-Soluble and Insoluble Macromolecules", Archives of Pathology, 67, 589-617, 1959) it is considered undesirable for pharmaceutical preparations administered by injection. This problem has been recognized and attempts of replacing polyvinyl alcohol with other emulsifiers can be found in the prior art, for example in US 4,384,975, wherein a carboxylic acid salt surfactant, e.g. sodium oleate was used to stabilise an oil-in-water emulsion. However, despite its drawbacks polyvinyl alcohol is still the most widely used surfactant. However, for the above-mentioned reasons it would be highly desirable to avoid the use of polyvinyl alcohol and other surfactants in microsphere preparations.

Solvent evaporation works well for hydrophobic drugs but for hydrophilic drugs, such as many peptides and proteins, the amount of incorporated drug can be low due to loss of drug to the aqueous phase which is used to extract the organic solvent. Attempts to circumvent this problem include modifying the hydrophilic drug into a less soluble form (WO 96/07399) increasing the viscosity of the inner aqueous solution containing the active drug in a process where a water-in-oil emulsion is first created and the organic solvent then extracted with water (US 4,652,441) and reducing the time available for diffusion (US 5,407,609).

Further, the use of the commonly employed organic solvents, like methylene chloride or ethyl acetate, often

results in loss of biological activity for sensitive drugs. Thus, for instance for proteins the three dimensional conformation which is required for biological activity is often lost. Attempts to circumvent this problem includes modification of the active substance into a more stable form (US 5,654,010 and WO 96/40074) keeping the temperature as low as possible during the process (WO 90/13780), and using different protein stabilisers (US 5,589,167, Cleland JL, Jones AJS, "Development of stable protein formulations for microencapsulation in biodegradable polymers". Proceedings of the International Symposium on Controlled Release of Bioactive Materials 1995;22:514-5). However, proteins are generally sensitive to organic solvents and reducing or eliminating the exposure is highly desirable.

Another disadvantage with the solvent evaporation method is the need for using high shear mixing in order to obtain small microspheres or nanospheres. This may result in degradation or conformational changes of the active substance, especially if it is a protein which is dependent on a three dimensional conformation for its biological activity. The use of high shear mixing is also energy consuming.

In connection with the prior art it can also be added that processes for preparing microspheres from polymers soluble in water are known from e.g. US 4,822,535 and US 5,578,709. In said processes two mutually immiscible aqueous liquid phases are used, of which one is solidified into microspheres. However, as said, these methods cannot be used for the preparation of microspheres from polymers that cannot be dissolved in water.

The present invention relates to a novel method of encapsulating active substances in biodegradable polymers by which the prior art disadvantages are eliminated or at least essentially reduced. For instance the invention makes it possible to obtain high incorporation efficiency

of the active substance in the biodegradable polymer and/or to accomplish smaller microparticles or even nanoparticles containing highly active doses of the active substances. Furthermore, the amounts of organic solvents are highly reduced. As compared to previously used methods the invention also enables a reduction of the energy input required to obtain micro- or nanoparticles.

#### OBJECTS OF THE INVENTION

One object of the invention is to provide a method of preparing controlled or sustained release particles having a high entrapment of water soluble substances, e.g. sensitive drugs, without the use of large volumes of organic solvents.

Another object is to provide a method wherein low energy mixing is utilized only, which is also advantageous in connection with sensitive substances.

Still another object is to provide a method by which small particle sizes, such as micro or even nano size particles, can be obtained in a simple way.

One other object is to provide a method by which the requirement for using PVA and other surfactants is eliminated.

Other objects of the invention should be clear to a person skilled in the art when reading the description above and below.

#### SUMMARY OF THE INVENTION

More specifically the present invention relates to a method of encapsulating an active substance in a biodegradable polymer, which method comprises:

- a) dissolving said biodegradable polymer in an organic solvent therefor;
- b<sub>1</sub>) dispersing said active substance in the organic solution obtained in step a), to provide a dispersion with the active substance as the inner phase thereof; or alternatively



b<sub>2</sub>) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a), to provide an emulsion with the active substance as the inner aqueous phase thereof;

5 and

c) subjecting the dispersion obtained in step b<sub>1</sub>), or alternatively the emulsion obtained in step b<sub>2</sub>), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase, so as to obtain  
10 micro- or nanoparticles having the active substance encapsulated therein.

Thus, according to one aspect of the invention there is provided a simple method of preparing micro- or nanoparticles containing a sensitive biologically active material, e.g. a protein, while using minimal amounts of  
15 organic solvent. It has surprisingly been found possible to replace the normally used organic solvent as the continuous or extraction phase by an aqueous solution of the non-toxic and pharmaceutically acceptable polymer polyethylene glycol (polyethylene oxide) as a continuous  
20 phase and as an extraction medium.

It has also been found that the uptake of active ingredient into the particles can be markedly improved by said use of polyethylene glycol in water or other aqueous  
25 solvent as outer(external) phase. The use of solvent evaporation techniques with an aqueous outer phase often results in poor encapsulation as water soluble polypeptides are distributed also to the external phase, especially when small microspheres are obtained. With the  
30 present invention high loading combined with small particle size can be obtained provided that the concentration of polyethylene glycol, and other conditions, are controlled such that the active substance is not distributed to the outer phase.

35 The microparticles can easily be washed and rinsed with water, which is an advantage as compared to the phase separation technique where large amounts of organic

solvents are used. Other surprising findings in connection with the use of polyethylene glycol/aqueous solvent as outer phase is that small sized particles are obtained even with low mixing forces and that no surfactants are  
5 needed.

The obtained microparticles are well suited for sustained release purposes and are especially adapted for oral or parenteral administration. When prepared with sizes or diameters of less than 10  $\mu\text{m}$ , and preferably  
10 0.5-3  $\mu\text{m}$ , they are also suitable for nasal or pulmonal administration to provide either local or systemic effect.

In addition to the unexpected findings referred to above it should also be noted that polyethylene glycol  
15 (PEG) is previously known per se to have unique properties for a variety of biotechnical and biomedical applications, which makes the present invention even more advantageous for biotechnical and biomedical applications.

These unique properties are e.g. summarized in Harris, J.M. (ed) Poly(ethylene glycol) chemistry: biotechnical and biomedical applications. 1992, Plenum Press,  
20 New York.

PEG has unique properties of major importance for its use in a variety of biotechnical and biomedical applications. One of these is its outstanding effectiveness  
25 in excluding other polymers from the volume it occupies in a water solution, which has been utilised to obtain rejection of proteins e.g. in liposomes and small particles with long circulation times after intravenous injection, hospitability to biological materials, non-  
30 immunogenicity and non-antigenicity. Another is the formation of aqueous two-phase systems with other polymers (Per Åke Albertsson, Partition of cell particles and macromolecules. Separation and purification of biomolecules, cell organelles, membranes, and cells in aqueous polymer  
35 two-phase systems and their use in biochemical analysis and biotechnology. Third Edition, 1986, John Siley &

Sons). PEG is non-toxic and generally harmless to proteins and cells. Of the numerous applications of PEG can be mentioned: (1) as a co-solvent for some drugs for injection, (2) as a volume-excluder to increase the concentration of e.g. proteins to induce crystallization, (3) as a part of aqueous two-phase systems used for e.g. purification of biological materials under mild conditions, (4) induction of cell fusion to obtain e.g. hybridomas used for production of monoclonal antibodies, (5) covering the surface of e.g. liposomes and nanoparticles to increase their residence time in the circulation, and (6) covalent attachment of PEG to proteins to obtain conjugates which are still biologically active but no longer immunogenic and antigenic; such PEG-protein adducts having been approved for parenteral use in humans.

PEGs are also sometimes referred to as poly(ethylene oxide) PEO, poly(oxyethylene) and polyoxirane. In general usage, poly(ethylene glycol) refers to molecular weights below 20000, and poly(ethylene)oxide refers to higher molecular weights polymers. In other words the term poly(ethylene glycol) as used in connection with the invention covers also poly(oxyethylene) and polyoxirane.

These objects as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the description following below.

#### DETAILED DESCRIPTION OF THE INVENTION

The active substances to be used in the method of the invention are preferable biologically active substances, e.g. drugs, such as proteins, peptides, polypeptides, polynucleotides, oligonucleotides, plasmids or DNA. Examples of protein drugs are growth hormone, erythropoietin, interferon( $\alpha$ , $\beta$ , $\gamma$ -type), vaccines, epidermal growth hormone and Factor VIII. Examples of peptide drugs are LHRH analogues, insulin, somatostatin, calcitonin, vasopressin and its derivatives.

In the case of proteins they can also be complexed with various substances, e.g. metals, amino acids, salts, acids, bases and amines, to decrease solubility or increase stability. They can further be prepared in the form of a pro-drug or PEG can be attached e.g. to the proteins to increase solubility or stability, modify pharmacokinetics or reduce immunogenicity.

Examples of non-protein drugs suitable for use in the method of the invention can be found for example in the following groups:

anti-tumor agents, antibiotics, anti-inflammatory agents, antihistamines, sedatives, muscle relaxants, antiepileptic agents, antidepressants, antiallergic agents, bronchodilators, cardiotonics, antiarrhythmic agents, vasodilators, antidiabetic agents, anticoagulants, hemostatics, narcotic agents and steroids.

The active substances which can be encapsulated in accordance with the method claimed are, however, not restricted to biologically active substances, non-biological substances can be encapsulated, e.g. pesticides, fragrances, flavouring agents, catalysts and herbicides.

The proper amount of active substance to be encapsulated is dependent on type of substance, duration time and desired effect, and is of course controlled to an amount that is in each specific case encapsulable by the method according to the invention. Generally said amount is chosen within the range of about 0,001% to 90%, preferably about 0,01 to 70%, more preferably about 0.1 to 45%, and most preferably about 0.1 to 40%, said percentages being by weight based on the weight of the final particles.

In the case of a drug the substance can be used per se or in the form of a pharmaceutical salt. When the drug has a basic group, such as amino groups, it can form salts with carbonic acid, hydrochloric acid, sulphuric acid, acetic acid, citric acid, methanesulfonic acid or

the like. When the drug has an acidic group, such as a carboxyl group, it can form salts with metals (e.g.  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ), organic amines (e.g. ethanolamine) or basic amino acids (e.g. arginine). The drug can further be precipitated using various means, optionally followed by size reduction, such as precipitation with divalent metals (e.g.  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ). The drug may also be crystallized.

The biodegradable polymer that can be used in the present invention is not limited to any specific material as long as it can be dissolved in an organic solvent and is slightly soluble or insoluble in the outer phase, e.g. poly(ethylene glycol)/aqueous phase and is otherwise suitable for the preparation of sustained release micro- or nanoparticles.

Preferably the biodegradable polymer used in the method claimed has a weight average molecular weight in the range of about 2000 to 200000, more preferably about 2000 to 110000.

Examples of biodegradable polymers are polyesters, poly- $\beta$ -hydroxybutyric acid, polyhydroxyvaleric acid, polycaprolactone, polyesteramides, polycyanoacrylates, poly(amino acids), polycarbonates and polyanhydrides.

A preferred biodegradable polymer is an aliphatic polyester, e.g. homo or copolymers prepared from  $\alpha$ -hydroxy acids, preferably lactic acid and glycolic acid, and/or cyclic dimers of  $\alpha$ -hydroxy acids, preferably lactides and glycolides.

When lactic acid/glycolic acid are used as the above-mentioned polymers, the composition or weight ratio (poly)lactic acid/(poly)glycolic acid is preferably about 99/1 to 35/65, more preferably 95/5 to 50/50. They may be used in the form of a copolymer or a mixture of these two or more polymers. The exact composition of the polymer depends on the desired release kinetics, especially the duration of release.

The organic solvent used in step A can be any solvent that is capable of forming an emulsion with a wa-

ter/PEG mixture, can be removed from the oil droplets through said water/PEG mixture and is capable of dissolving the biodegradable polymer. In other words the solvent should be immiscible, or essentially immiscible, but  
5 slightly, or very slightly, soluble in said water/PEG mixture. Examples of suitable solvents are ethyl acetate, dichloromethane, methyl ethyl ketone and methyl isobutyl ketone. These solvents can be used alone or in combinations.

10 The inner aqueous phase may contain agents for controlling the stability and, if desired, the solubility of the biologically active substance. Such agents may be pH controlling agents and stabilizers for drugs or other active substances.

15 As can be gathered from the above-mentioned the method according to the invention can be utilized to encapsulate water soluble as well as water insoluble active substances.

Examples of embodiments of these two cases will now  
20 be presented below.

The encapsulation method, as exemplified by a water soluble drug, such as a peptide or protein drug can comprise the following steps. The drug solution is prepared in any conventional way and optionally while using pH  
25 controlling or drug stabilizing agents. This aqueous solution of the drug, which is to form the inner aqueous phase, is poured into an external (oil) phase containing a biodegradable polymer dissolved in a suitable organic solvent and the mixture is emulsified to provide a W/O  
30 emulsion. The emulsification can be prepared using conventional emulsification techniques, such as, propeller mixing, turbine mixing, ultrasonication or use of static mixers.

If the active substance is to be dispersed directly  
35 in the polymer solution, without being dissolved in water, the drug should have a suitable particle size. A suitable particle size is about 0.5-20  $\mu\text{m}$ , preferably

0.5-10  $\mu\text{m}$ , such as 0.5-3  $\mu\text{m}$ . Otherwise, the dispersion step can be carried out as described above for the emulsification step.

The resulting W/O emulsion/dispersion is then subjected to an encapsulation operation. The W/O emulsion/dispersion is added to an aqueous solution containing polyethylene glycol. The polyethylene glycol/aqueous solution is stirred during the addition of the active substance/polymer solution. The W/O emulsion/dispersion can also be mixed with the polyethylene glycol solution by using motionless mixers.

Typically the molecular weight of the polyethylene glycol is within the range of about 1000 to 40000 Da, preferably 5000 to 35000 Da. Depending on said molecular weight, and the properties of the substance to be encapsulated, the concentration of polyethylene glycol is controlled within the range of 20-80% (w/w), preferably 20-60% (w/w), such as 30-55% (w/w) or 30-50% (w/w). In other words a relatively high PEG concentration is used in the outer phase, to obtain a stable emulsion and to prevent diffusion of active ingredient from the droplets/particles. The determination of the optimal concentration can be made by experimentation that is relatively straightforward to someone skilled in the art.

The particles thus formed are generally collected by centrifugation or filtration and rinsed with distilled water or suitable aqueous buffers, several times to remove the excess of polyethylene glycol from the surfaces. To prevent aggregation during the washing and drying procedure, mannitol, Tween 80, or other suitable substances, may be added to the rinsing water. The particles thus obtained can then be dried by conventional means, for instance in vacuum or by a streaming nitrogen gas flow or by lyophilization or air suspension drying.

The particle sizes of the particles obtained by the invention are dependent on the desired uses of said particles as is well known within this technical field.

Thus, for instance, when the particles are intended for injection, the particle size should satisfy the dispersibility and needle passage requirements. Furthermore, the particles can be handled or treated in any manner previously known to a person skilled in the art. Thus, a controlled release injectable preparation of said particles can e.g. be dispersed with a suspending agent, containing e.g. mannitol, polysorbate 80, or sodium carboxymethylcellulose.

Other embodiments of the method according to the invention are defined in sub-claims or in the Examples presented below.

According to a second aspect of the invention there are also provided sustained release micro or nanoparticles per se containing an active substance encapsulated in a biodegradable material, which particles are obtainable by a method as claimed in any one of the method claims.

Thus, preferable embodiments thereof are the same as those embodiments which are described in connection with the method. Especially preferable are, however, particles which are adapted for oral, parenteral, nasal or pulmonal administration of the active substance.

Furthermore, for the manufacture of pharmaceutical preparations for oral administration, the microspheres prepared by the method described may be formulated with an excipient (e.g. lactose, sucrose, starch etc.), a disintegrant (e.g. starch, calcium carbonate, etc.), a binder (e.g. starch, gum arabic, carboxymethylcellulose, polyvinylpyrrolidone, etc.) and/or a lubricant (e.g. talc, magnesium stearate, polyethylene glycol etc.) and the resulting composition can be compression-molded in conventional manner. The particles can also be filled into gelatine capsules.

FIGURE



In the accompanying drawing figure the results of in vitro release tests are presented for particles obtained by the method of the present invention as well as particles obtained in line with the prior art.

5       The manufactures of said particles and the test method are described in Examples 1-5 and the results are presented as cumulative release in % versus time in days.

10       In this context it can also be added that the release profile can be controlled by factors well known to anyone skilled in the art, e.g. the composition of the polymer used for encapsulating the active material, the solubility of the material, addition of substances affecting the solubility of the active material and/or degradation of the polymer, the amount of active material  
15       in the microparticles and the size of the microparticles.

#### EXAMPLE 1

20       The following procedure was used to encapsulate bovine serum albumin (BSA) in PLGA (poly(DL-lactide-co-glycolide)). First a polymer solution was prepared by dissolving 0.47 g of PLGA (RG504H, Boehringer Ingelheim) in 3 ml of ethyl acetate in a test tube. Then, 44 mg of BSA, (bovine serum albumin; Sigma A-0281) was dissolved in 300 µl of 10 mM Na-phosphate buffer pH 6.4. The BSA  
25       solution was added to the polymer solution and the BSA was homogenously dispersed in the polymer solution by vortex mixing (VF2, IKA-WERK) for one minute. The dispersion was placed in a 5 ml syringe with an 18 G needle.

30       A 500 ml beaker containing 300 ml of 40%(w/w) polyethylene glycol 20000 was fitted with a 4-bladed propeller stirrer. The BSA/polymer dispersion was transferred to the beaker by slowly injecting the BSA/polymer dispersion into the PEG solution. The stirrer speed was then reduced and the mixture was left standing overnight.

35       The stirrer speed was set at 8 again and then 400 ml of deionized water were added to reduce the viscosity in order to enable filtration. The suspension was then fil-

tered using a Millipore membrane filter, Type DV, pore size 0.65  $\mu\text{m}$ , washed with water (3x 300 ml) and dried in vacuum overnight.

The resulting microparticles were spherical with a particle diameter of 10-50  $\mu\text{m}$  and contained 6.3 % of BSA (w/w).

The resulting microparticles were then subjected to an in vitro release test in 30 mM sodium phosphate pH 7.4 at 37°C, with intermittent agitation. The studies were conducted by suspending 40 mg of microspheres in 1.5 ml of buffer. At specified time points, 1 ml of the buffer was withdrawn and replaced with fresh buffer. The results are shown in figure 1. Sustained release of BSA was achieved for 28 days as is shown in figure 1.

#### EXAMPLE 2

The same procedure was performed as in example 1 except that 2% (w/w) polyvinyl alcohol (PVA, mw=22000, Fluka) in water was used instead of the polyethylene glycol solution.

The resulting microspheres had a particle diameter of 1-2 mm and contained 7.0% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1. Sustained release for about 2 days was achieved with this formulation. The large size would not have permitted injection using acceptable needles.

#### EXAMPLE 3

The same procedure was performed as in example 1 except that the an Ystral homogenizer was used instead of said stirrer when adding the BSA/polymer dispersion. After addition of the BSA/polymer dispersion the homogenizer was replaced by the 4-bladed propeller stirrer.

The resulting microspheres had a particle diameter of 1-5  $\mu\text{m}$  and contained 5.5% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1.

EXAMPLE 4

The same procedure was performed as in example 2 except that the an Ystral homogenizer was used instead of a stirrer when adding the BSA/polymer dispersion.

The resulting microspheres had a mean particle diameter of 10-40  $\mu\text{m}$  and contained 5.8% of BSA. An in vitro release test was conducted as in example 1 and the results are shown in figure 1. Similar dissolution profiles were obtained for the preparations in examples 3 and 4 even though the size of the particle in example 3 was much smaller.

EXAMPLE 5

The same procedure was performed as in example 1 except that an ultrasonic bath (Transsonic 470/H, Elma) was used after the vortex mixing in order to obtain a finer water-in-oil emulsion. The BSA/polymer dispersion was sonicated for 1 minute.

The resulting microspheres had a mean particle diameter of 10-50  $\mu\text{m}$  and contained 6.8% of BSA. An in vitro test was conducted as in example 1 and the results are shown in figure 1. Sustained release for 28 days was achieved. This shows that a more efficient emulsification of the inner aqueous phase results in a lower rapid initial release (burst) during the first days.

EXAMPLE 6Preparation of BSA loaded microspheres

The following procedure was used to encapsulate Bovine Serum Albumin (BSA) in PLGA microspheres.

First a polymer solution was prepared by dissolving 0.126 g of polymer (Resomer 504H, Boehringer Ingelheim) with 0.734 of ethyl acetate in a test tube. Then 15 mg of BSA (Sigma A-0281) were dissolved in 100  $\mu\text{l}$  of 10 mM sodium phosphate pH 6.4.

The BSA solution was mixed with the polymer solution

by vortex mixing (VF2, IKA-WERK) for one minute. The solution was withdrawn into a 2 ml syringe with a 21G needle. A 200 ml beaker containing 50 ml of 40% (w/w) polyethylene glycol 20000 was fitted with a 4-bladed propeller stirrer. The BSA/polymer dispersion was slowly injected into the PEG solution during stirring at 240 rpm. The stirring speed was increased to 400 rpm for 10 seconds then the stirring speed was 60 rpm for one minute. The mixture was left standing unstirred for 4 hours.

200 ml of water were then added before filtration. The microsphere suspension was filtered using a Millipore membrane filter, Type DV, pore size 0.65  $\mu$ m, washed with water and then freeze-dried overnight.

The resulting microparticles were spherical with a particle diameter of 10-50  $\mu$ m and contained 9.7% of BSA (92% yield).

#### EXAMPLE 7

##### Preparation of Lactoglobulin loaded microspheres

The same procedure was performed as in example 6, except that 15 mg of Lactoglobulin (Sigma L-0130) in 100  $\mu$ l 10 mM sodium phosphate pH 6.4 were used for encapsulation.

The resulting microparticles were spherical with a particle diameter of 10-100  $\mu$ m and contained 9.9% of lactoglobulin (93% yield).

#### EXAMPLE 8

##### Preparation of Triptorelin loaded microspheres

The same procedure was performed as in example 6, except that 15 mg of Triptorelin pamoate (Bachem) were emulsified directly in the polymer solution by vortex mixing for one minute. The particle size of triptorelin particles was about 2-4  $\mu$ m.

The resulting microparticles were spherical with a particle diameter of 20-100  $\mu$ m and contained 6.3% of Triptorelin (59% yield).

EXAMPLE 9Preparation of Desmopressin loaded microspheres

The same procedure was performed as in example 6,  
5 except that 15 mg Desmopressin acetate in 100 µl of 10 mM  
sodium phosphate pH 6.4 were used for encapsulation.

The resulting microparticles were spherical with a  
particle diameter of 10-50 µm and contained 8.3% of Des-  
mopressin (78% yield).

10

EXAMPLE 10Preparation of Insulin loaded microspheres

The same procedure was performed as in example 6,  
except that 15 mg Insulin (Sigma I-5500) were emulsified  
15 directly in the polymer solution by vortex mixing for one  
minute. The particle size of the insulin particles was  
about 5-10 µm.

The resulting microparticles were spherical with a  
particle diameter of 10-50 µm and contained 9.3% of Insu-  
lin (88% yield).

20

EXAMPLE 11Preparation of DNA loaded microspheres

The same procedure was performed as in example 6,  
25 except that 100 µl of Herring Sperm DNA (Promega) (10  
mg/ml) were used for encapsulation.

The resulting microparticles were spherical with a  
particle diameter of 10-50 µm and contained 0.07% of DNA  
(10% yield).

30

EXAMPLE 12Preparation of Bovine Serum Albumin in 50% PEG 10k

The same procedure was performed as in example 6,  
except that 50% of PEG 10k was used as the external  
35 phase.

The resulting microparticles were spherical and con-  
tained 1.77% of BSA. This should be compared to 6.3% in

example 1.

EXAMPLE 13

Preparation of Bovine Serum Albumin in 30% PEG 35k

5       The same procedure was performed as in example 1 except that 30% of PEG 35k was used as the external phase.

      The resulting microparticles were spherical and contained 5.42% of BSA. This should be compared to a core load of 6.3% in example 1.

CLAIMS

1. A method of encapsulating an active substance in a biodegradable polymer, which comprises:

- 5 a) dissolving said biodegradable polymer in an organic solvent therefor;
- b<sub>1</sub>) dispersing said active substance in the organic solution obtained in step a), to provide a dispersion with the active substance as the inner phase thereof; or alternatively
- 10 b<sub>2</sub>) emulsifying said active substance, dissolved in water or other aqueous solvent therefor, in the organic solution obtained in step a), to provide an emulsion with the active substance as the inner aqueous phase thereof; and
- 15 c) subjecting the dispersion obtained in step b<sub>1</sub>), or alternatively the emulsion obtained in step b<sub>2</sub>), to an encapsulation operation with an aqueous polyethylene glycol solution as a continuous phase, such that micro- or nanoparticles having the active substance encapsulated
- 20 therein are obtained.

2. A method according to claim 1, wherein the microencapsulation operation in step c) is performed in the presence of an aqueous polyethylene glycol solution having a polyethylene glycol concentration within the range

25 of 20-80% (w/w), preferably 20-60% (w/w), such as 30-55% (w/w) or 30-50% (w/w).

3. A method according to any one of claims 1 and 2, wherein the polyethylene glycol has a molecular weight of about 1000 to 40000 Da, preferably about 5000 to 35000

30 Da.

4. A method according to any one of claims 1, 2 and 3, wherein the encapsulation operation in step c) is performed by adding the dispersion obtained in step b<sub>1</sub>), or alternatively the emulsion obtained in step b<sub>2</sub>), to said

35 aqueous polyethylene glycol solution while subjecting last-mentioned aqueous solution to a stirring and/or ho-

mogenization operation.

5 5. A method according to claim 4, wherein the stirring and/or homogenization operation is performed by a low intensity and/or low energy process, e.g. propeller mixing or the use of motionless mixers.

6. A method according to any one of the preceding claims, wherein said encapsulation operation in step c) is performed in the absence of any surfactant.

10 7. A method according to any one of the preceding claims, wherein said biodegradable polymer is insoluble, or slightly soluble, in the aqueous polyethylene glycol solution used in step c), preferably an aliphatic polyester.

15 8. A method according to any one of the preceding claims, wherein said biodegradable polymer has a weight average molecular weight in the range of about 2000 to 200 000, preferably about 2000 to 110 000.

20 9. A method according to any one of the preceding claims, wherein said biodegradable polymer is selected from homo or copolymers prepared from  $\alpha$ -hydroxy acids, preferably lactic acid and glycolic acid, and/or cyclic dimers of  $\alpha$ -hydroxy acids, preferably lactides and glycolides.

25 10. A method according to claim 9, wherein a copolymer of lactic acid/glycolic acid or a mixture of polylactic acid/polyglycolic acid is used as said biodegradable polymer, the weight ratio of (poly)lactic acid/(poly)glycolic acid being within the range of about 99/1 to 35/65, preferably 95/5 to 50/50.

30 11. A method according to any one of the preceding claims, wherein said organic solvent used in step a) is immiscible or essentially immiscible with said aqueous polyethylene glycol solution used in step c), but slightly or very slightly soluble therein, and capable of dissolving said biodegradable polymer, and is preferably  
35 selected from ethyl acetate, dichloromethane, methyl ethyl ketone and/or methyl isobutyl ketone.



12. A method according to any one of the preceding claims, wherein the active substance which is dispersed in step b<sub>1</sub>) has a particle size within the range of about 0.5-20  $\mu\text{m}$ , preferably 0.5-10  $\mu\text{m}$ , more preferably 0.5-3  $\mu\text{m}$ .

13. A method according to any one of the preceding claims, wherein said active substance is a biologically active substance, which is preferably selected from proteins, (poly)peptides, (poly)nucleotides, plasmides and DNA.

14. A method according to claim 13, wherein said biologically active substance is selected from growth hormone, erythropoietin, interferon ( $\alpha, \beta, \gamma$ -type), vaccine, epidermal growth hormone, Factor VIII, LHRH analogue, insulin, macrophage colony stimulating factor, granulocyte colony stimulating factor and interleukin.

15. A method according to any one of claims 1-12, wherein said active substance is a biologically active substance in the form of a non-protein drug selected from the following groups:

anti-tumor agents, antibiotics, anti-inflammatory agents, antihistamines, sedatives, muscle relaxants, antiepileptic agents, antidepressants, antiallergic agents, bronchodilators, cardiotonics, antiarrhythmic agents, vasodilators, antidiabetic agents, anticoagulants, hemostatics, narcotic agents and steroids.

16. A method according to any one of claims 1-12, wherein said active substance is a non-biological substance, which is preferably selected from pesticide, fragrance, flavouring agent, catalyst and herbicide.

17. A method according to any one of the preceding claims, wherein the amount of said active substance is in the range of about 0.001% to 90%, preferably about 0.01% to 70%, more preferably about 0.1 to 45%, and most preferably about 0.1 to 40%, said percentage being by weight based on the weight of the final particles.

18. A method according to any one of the preceding

claims, wherein the particles obtained in step c) are separated from said continuous phase, preferably by centrifugation or filtration followed by rinsing with water or other aqueous medium, and dried or allowed to dry, for instance in a vacuum, in the presence of a nitrogen gas flow, by lyophilisation or by air suspension drying.

19. A method according to any one of the preceding claims, wherein step c) is performed such that the particles obtained are microspheres or capsules or nanospheres or capsules.

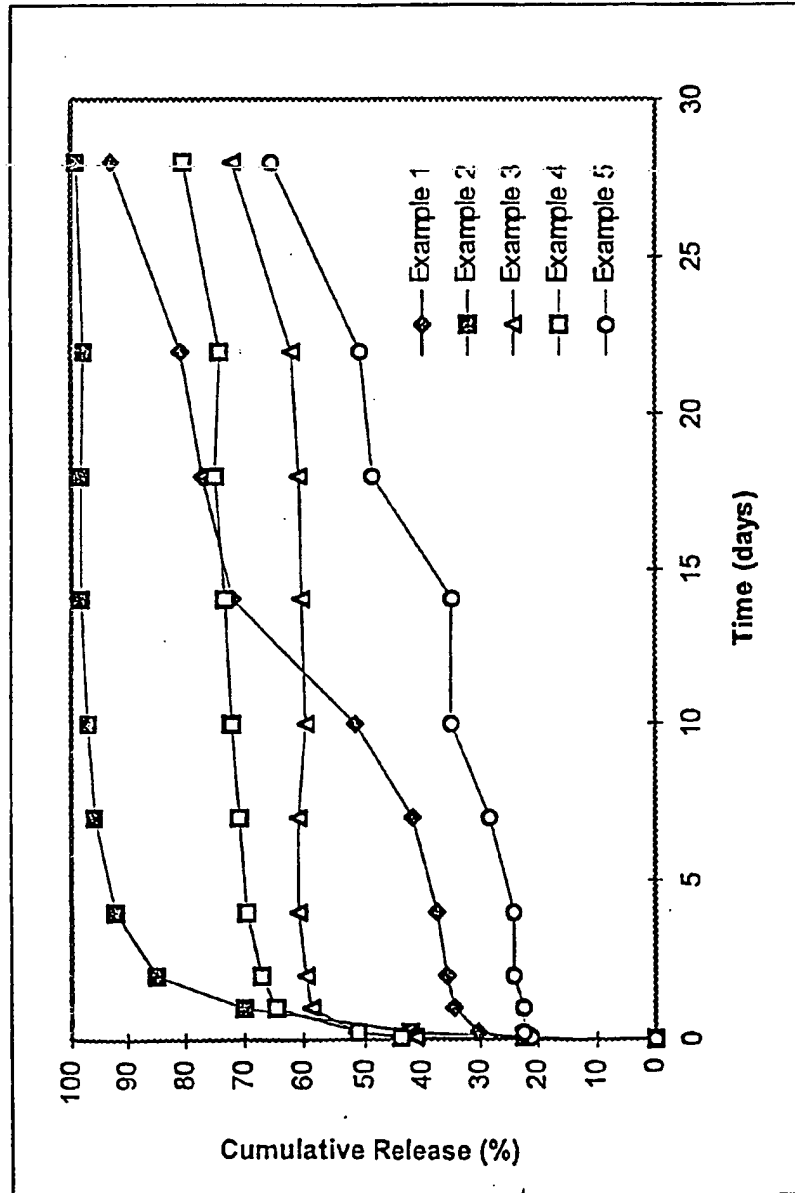
20. A method according to claim 19, wherein said particles have a mean diameter in the range of 10-200  $\mu\text{m}$ , preferably 20-100  $\mu\text{m}$ .

21. Sustained release micro or nanoparticles containing an active substance encapsulated in a biodegradable polymer, obtainable by a method according to any one of claims 1-20.

22. Particles according to claim 21, which are suitable for parenteral, nasal, pulmonal or oral administration of said active substance.

1/1

FIGURE



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01717

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 9/14, A61K 9/50, B01J 13/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAPLUS, EMBASE, USPATFULL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                            | Relevant to claim No. |
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| A         | US 4652441 A (CHIROAKI OKADA ET AL), 24 March 1987<br>(24.03.87), claims<br>--                                | 1-22                  |
| A         | US 5407609 A (THOMAS R. TICE ET AL), 18 April 1995<br>(18.04.95), column 3, line 49 - column 7, line 27<br>-- | 1-22                  |
| A         | US 4568559 A (ELIE S. NUWAYSER ET AL),<br>4 February 1986 (04.02.86), claims<br>--                            | 1-22                  |

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"Z" document member of the same patent family

Date of the actual completion of the international search

12 February 1999

Date of mailing of the international search report

22 -02- 1999

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01717

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------|-----------------------|
| A         | EP 0052510 A2 (SYNTEX INC.), 26 May 1982<br>(26.05.82)<br><br>--<br>-----          | 1-22                  |

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Information on patent family members

02/02/99

International application No.

PCT/SE 98/01717

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